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IgG INTERCEPT™ MICRO-PLATE EIA for use with Intercept™ Drugs of Abuse (DOA) Oral Fluid Specimens

14034 (10/01)

INTENDED USE

The OraSure Technologies, Inc. (OTI) IgG Intercept™ MICRO-PLATE EIA is intended for use in the qualitative determination of human IgG in oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. This test ensures that specimens contain sufficient human IgG as a measure of sample adequacy in the Intercept™ DOA Collection Device. ***THIS TEST IS INTENDED FOR INSURANCE RISK ASSESSMENT ONLY.***

PRINCIPLE OF THE ASSAY

The OTI IgG Intercept™ MICRO-PLATE EIA is a sandwich enzyme immunoassay. The solid phase antibody (anti-human IgG) is incubated with a diluted sample. If the sample contains human IgG, the IgG will be captured on the solid phase. After sample incubation and washing, the enzyme-labeled antibody (anti-human IgG with enzyme label) is added. The labeled antibody will attach to, or sandwich, human IgG bound to the solid phase. After a second wash step to remove unbound conjugate, substrate is added to react with the enzyme, resulting in a color change proportional to the amount of bound human IgG. This reaction is then stopped and the results are measured spectrophotometrically.

KIT COMPONENTS	Catalog No. 11321B	Catalog No. 11321C
	480 Test Kit	9600 Test Kit
	Min. Qty.	Min. Qty.
Microplate - Rabbit anti-human antibody immobilized on a polystyrene plate supplied in dry form.	5	100
Enzyme Conjugate - Horseradish peroxidase labeled with rabbit anti-Human IgG (H+L) diluted in a liquid protein matrix.	60 mL	1 L
Sample Diluent - Each bottle contains protein matrix of bovine serum with protein stabilizers.	60 mL	1 L
Substrate Reagent - Each bottle contains 3,3',5,5'- tetramethylbenzidine.	60 mL	1 L
Stopping Reagent - Each bottle contains 2.0 N sulfuric acid. Treat this solution as corrosive.	60 mL	1 L
Oral Fluid Negative Calibrator - Oral Fluid Diluent assayed to be negative for Human IgG.	2 mL	16 mL
Oral Fluid Negative Control - Oral Fluid Diluent containing 0.25 µg/mL Human IgG.	2 mL	16 mL
Oral Fluid Cutoff Calibrator - Oral Fluid Diluent containing 0.5 µg/mL Human IgG.	2 mL	16 mL
Oral Fluid Positive Control - Oral Fluid Diluent containing 2.0 µg/mL Human IgG.	2 mL	16 mL
Oral Fluid Positive Control - Oral Fluid Diluent containing 4.0 µg/mL Human IgG.	2 mL	16 mL

WARNINGS AND PRECAUTIONS

1. Gloves and protective eyewear should be worn when performing this assay.
2. The handling of food or drink near the kit reagents is **NOT** recommended.
3. Handle all reagents carefully. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
4. Do **NOT** mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
5. Keep all containers closed when not in use to avoid microbial contamination.
6. Do **NOT** add sodium azide to samples as a preservative!
7. Do **NOT** use reagents past the expiration date.
8. Do **NOT** mix reagents from different kits or manufacturers.
9. Do **NOT** freeze reagents.
10. Keep all reagents out of direct sunlight whenever possible.
11. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY OF THE OTI IgG INTERCEPT™ MICRO-PLATE EIA

Store all reagents at 2-8 °C until the expiration date indicated on the kit label.

INTERCEPT™ DOA SPECIMEN PROCESSING PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Tubes suitable for centrifuging Intercept™ DOA Specimen Vials.
2. Centrifuge capable of 600 - 800 x g.

PROCEDURE (Refer to Intercept™ DOA Collector package insert for more information)

1. Record the specimen identification number from the Intercept™ DOA Specimen Vial.
2. Ensure that the specimen is within acceptable dating for testing, i.e. ≤ 21 days from the time of collection.
3. Hold the vial upright with the tip pointed up.
4. Move the pad away from the vial tip by gently tapping the vial.
5. Break the pointed tip of the vial off with your thumb.
6. Place a tube over the vial and invert the tube and vial.
7. Centrifuge at 600 - 800 x g for 15 minutes.
8. Assay or store the resulting eluate according to the procedures described herein.
9. A minimum of 0.7 mL of the eluate sample is required. This can be determined by centrifugation of the samples into graduated containers or by direct pipetting with a calibrated pipet adjusted to the specified volume.
10. If the minimum volume requirement is not met, a new sample should be collected. If this is not possible, a warning should accompany any data generated indicating that an insufficient amount of sample was collected and that this may affect the accuracy of the final result.

ASSAY PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (15, 85 and 100 microliters) with tips.
2. Micro-plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Micro-plate washer.
4. Intercept™ DOA eluate sample(s) - 0.7 mL minimum.

PROCEDURE -- Note: Allow reagents and samples to come to room temperature (15-27°C) before use.

1. At the discretion of the operator, all samples, calibrators, and controls may be tested in duplicate. The inclusion of calibrators and controls is recommended on each new plate.
2. Simultaneously, add 15 microliters of sample and 85 microliters of Sample Diluent to each microtiter plate well. Gently tap or shake plate for 10 seconds to assure proper mixing of sample and diluent.
3. Incubate for 30 minutes at room temperature (15-27°C).
4. Wash the plate using a suitable plate washer. Wash each well six (6) times with 300 microliters of distilled water. After washing, blot on toweling to remove residual water.
5. Add 100 microliters of Enzyme Conjugate to each well and incubate for 30 minutes at room temperature (15-27°C) in the dark.
6. Wash the plate using a suitable plate washer. Wash each well six (6) times with 300 microliters of distilled water. After washing, blot on toweling to remove residual water.
7. Add 100 microliters of Substrate Reagent to each well and incubate for 30 minutes at room temperature (15-27°C) in the dark.
8. Add 100 microliters of Stopping Reagent to each well. Gently tap or shake plate for 10 seconds to assure proper mixing of Stopping Reagent.
9. Measure the absorbance at a dual wavelength of 450 and 630 nm within 5 minutes of stopping the reaction.

INTERPRETATION

Positive Result: Any sample with an absorbance greater than or equal to the Oral Fluid Cutoff Calibrator is considered a sufficient human specimen.

Negative Result: Any sample with an absorbance less than the Oral Fluid Cutoff Calibrator is considered an inadequate specimen.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. It is recommended that duplicate sample results which differ by more than 10% be retested.

QUALITY CONTROL

OTI supplies Positive and Negative Controls to monitor the daily performance of the OTI μ G Intercept™ MICRO-PLATE EIA. The Oral Fluid Negative Control must have an absorbance less than the Oral Fluid Cutoff Calibrator. The Oral Fluid Positive Control must have an absorbance greater than the Oral Fluid Cutoff Calibrator. The use of controls from other commercial vendors is acceptable as long as they do NOT contain sodium azide.

LIMITATIONS OF THE PROCEDURE

The assay is designed for use with oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. Other samples may produce variable results and their use is not recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS OF INTERCEPT™ DOA SPECIMENS

Analytical Sensitivity/Limit Of Detection - The Limit of Detection (LOD) was defined from the signal-to-noise ratio at the zero-drug concentration as the mean zero absorbance (A_0) minus the noise times three ($LOD = A_0 - 3SD$). The LOD was determined by obtaining the average absorbance value for 24 readings of Oral Fluid Diluent and calculating the standard deviation (SD) and 3SD of the absorbance. The absorbance value minus 3SD was then extrapolated from the curve and represents the sensitivity of the assay. The LOD was calculated to be 0.0015 μ g/mL.

Precision - Precision was evaluated for the OTI IgG Intercept™ MICRO-PLATE EIA by analyzing five levels of calibrators and controls. Inter-assay precision for the Negative Calibrator, Negative Control and Cutoff Calibrator was determined over a five-day period with twenty-four (24) samples tested at each calibrator level per day. Inter-assay precision for the positive controls was determined over a five-day period with twelve (12) samples tested at each level per day. Intra-assay precision was determined by analyzing the data from the first day. The IgG concentration levels were 0, 0.25, 0.5, 2.0 and 4.0 µg/mL.

IgG (mg/mL)	INTRA-ASSAY % CV	INTER-ASSAY % CV
0	12.8	22.5
0.25	4.2	6.6
0.5	3.9	7.7
2.0	3.4	7.5
4.0	3.8	6.4

Analytical Specificity/Cross-Reactivity - Canine IgG and Feline IgG were spiked into Oral Fluid Diluent at various concentrations up to 1,000 µg/mL and tested for cross-reactivity. Neither material produced an absorbance reading greater than or equal to the 0.25 µg/mL Negative Control.

It is possible that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedural errors.

REFERENCES

1. Intercept™ Oral Specimen Collection Device, Package Insert. Manufactured by OraSure Technologies, Inc., Beaverton, OR 97008.

Note: *Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.*
